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Conidial fungi from the semi-arid Caatinga biome of Brazil. New species and new records of *Helicosporium*

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Abstract —A new helicosporous fungus, *Helicosporium vesiculiferum*, is described based on morphological characters. It was found on a decaying twig of an unidentified dicotyledonous plant from the semi-arid region, Northeast of Brazil. The new species is illustrated with light micrographs and scanning electron microscopy. *Helicosporium vesiculiferum* is characterized by the presence of vesicles on apex of the conidiophores. Seven helicosporous species are briefly described, of which *H. virescens* and *H. aureum* are new records from Brazil, *H. panacheum* and *H. gracile* are reported for the first time in South America, and *H. nizamabadense* is new for the American continent. A key to all *Helicosporium* species is included.

Key words — anamorphic fungi, taxonomy

Introduction

Helicosporous fungi are defined by their coiled asexual spores (Tsui & Berbee 2006) and are considered aero-aquatic (Dix & Webster 1995). They are commonly found on substrates with abundant water (Mercado-Sierra 1982). In the present study these fungi were found in a semi-arid region, demonstrating the plasticity and survival capacity of helicosporous fungi in an inhospitable environment with low water availability and high temperatures.

Since Link (1809) described the first helicosporous species, *Helicomyces roseus,* several studies have been devoted to this group (e.g., Morgan 1892;

Linder 1929; Moore 1953, 1955; Goos 1980, 1985a,b,c, 1986, 1989, 1990). There are about 240 described species in 45 genera (Saccardo 1886, Linder 1929, Goos 1987, Zhao et al. 2007).

Phylogenetic studies are clarifying the relationship between the major genera of helicosporous fungi and their teleomorph connections. Tsui et al. (2006) analyzed rDNA of species of *Helicomyces* Link, *Helicoma* Corda, and *Helicosporium* Nees and verified that although all were related to *Tubeufia* Penz. & Sacc. sensu M.E. Barr, they are not monophyletic. Tsui & Berbee (2006) confirmed and extended the polyphyletism to *Helicodendron* Peyronel and *Helicoon* Morgan and suggested that the convergent form of the conidia is a possible adaptation to dispersion in aquatic environments.

Helicosporium is characterized by the presence of long and conspicuous conidiophores and conidia that are relatively thin-walled and hygroscopic (Goos 1989). The species are mainly distinguished by the morphology of conidia (diameter, filament width and number of coils), conidiophores, conidiogenous cells, and colony color (Goos 1989). Currently 21 species are included in the genus (Zhao et al. 2007)

Up to now, nine helicosporous fungi have been reported for Brazil: *Dichotomophthoropsis nymphaearum* (F.V. Rand) M.B. Ellis (Cavalcanti & Milanez 2007), *Hiospira jambosae* Bat. et al. (Batista et al. 1964), *Helicoma bambusae* Henn. (Hennings 1902), *H. palmarum* G.Z. Zhao et al. (Samuels et al. 1978, as *Helicomyces* sp.), *Helicomyces roseus* Link (Goos 1985b), *Helicosporium griseum* and *H. guianense* Linder (CBS 2009), *H. pannosum* (Sivanesan 1984), and *Xenosporium berkeleyi* (M.A. Curtis) Piroz. (Mendes et al. 1998, as *Xenosporella berkeleyi* (M.A. Curtis) Linder).

Previous studies of leaf litter conidial fungi from Caatinga biome have demonstrated the richness of this region (Castañeda-Ruiz et al. 2006; Cruz et al. 2007 a, b; Gusmão et al. 2008; Leão-Ferreira et al. 2008). During a recent survey an interesting specimen of *Helicosporium* was found. It does not resemble hitherto described species and is proposed here as new.

Materials and methods

The Program of Research in Biodiversity of the Brazilian semi-arid (PPBIO/Semi-árido) is investigating the biodiversity in Caatinga biome. This biome is considered part of a phytogeographical unit of South America called the neotropical seasonally dry tropical forests (Pennington et al. 2000, Prado 2000). It is characterized by high annual average temperatures (26–28ºC) and a low precipitation (300–1000 mm/year) that is usually concentrated within 3–5 months; droughts are common (Sampaio 1995).

Samples of plant litter were collected in separate paper bags and taken to the laboratory where each was incubated at 25ºC in Petri dishes within 170 L plastic chambers containing 200 ml sterile water and 2 ml glycerol. The plant material was screened at regular intervals for microfungi. Mounts were prepared in polyvinyl alcohol-glycerol

(8 g per 100 ml H_20, 5 ml glycerol) and deposited in the Herbarium of Universidade Estadual de Feira de Santana (HUEFS).

Taxonomy

Helicosporium vesiculiferum A.C. Cruz & Gusmão **sp. nov**. Figs.1–9

MycoBank MB512999

Coloniae in substrato naturali effusae, albae. Mycelium partim superficiale et partim in substrato immersum, ex hyphis ramosis, septatis, pallide brunneis laevibus, 3–6 µm latis compositum. Conidiophora ex hyphis oriunda, macronematosa, mononematosa, 4–12-septata, simplicia vel interdum ramosa prope basem, erecta, recta vel flexuosa, laevia, brunnea, pallide brunnea vel subhyalina ad apicem, 32.5–155 × 3–7.5 µm; vesicula in apice, plerumque praesentia, globosa, subhyalina, 5–9 × 4.5–8 µm; rami 1–2-septata, laeves, brunnea vel pallide brunnea ad apicem, 10–35(–80) × 2.5–6 µm. Cellulae conidiogenae polyblasticae, in conidiophoris incorporatae, intercalares et terminales, cylindricae, laevia, brunneae vel pallidae brunneae; denticulae 0.8–2 × 0.7–2 µm. Conidia holoblastica, solitaria, sicca, acropleurogena, 10–15-septata, helicoidea, laevia, hyalina, 11–18 µm diam, filamenta conidica 1–1.5 µm crassa, in 2–3.5 spiris convolute.

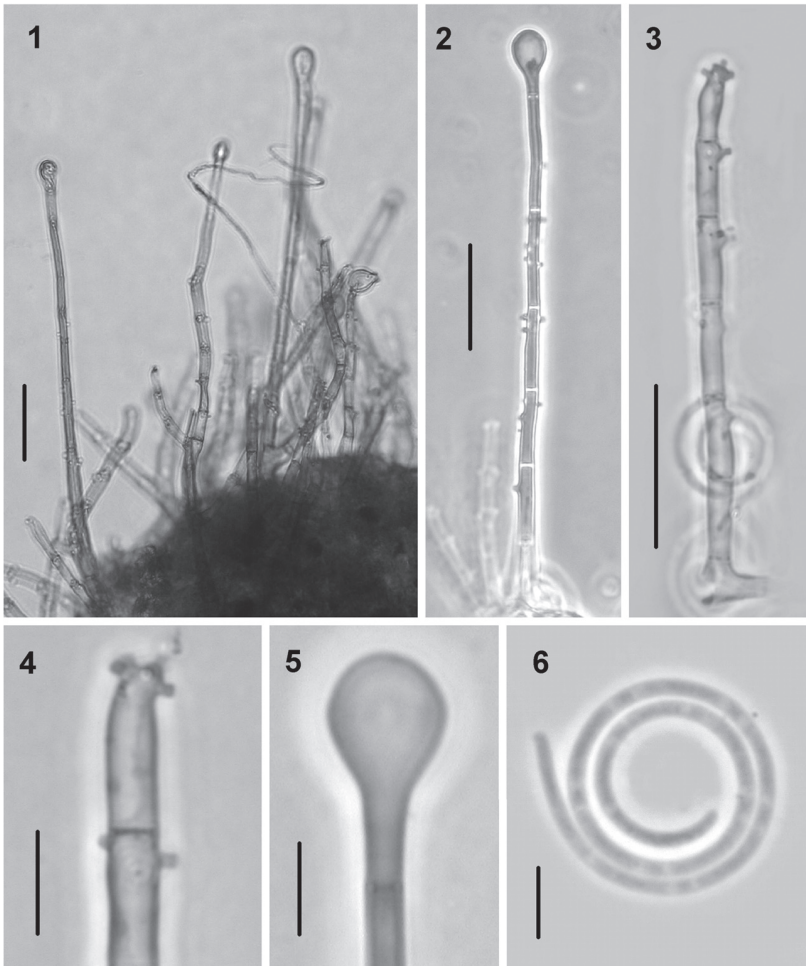
HOLOTYPUS: BRAZIL. Bahia: Senhor do Bonfim, in ramulis emortuis angiospermae ignotae, 25.IX.2006, A.C.R. Cruz, HUEFS 129360.

Etymology: Latin, *vesiculiferum* = carrying vesicles.

Colonies on natural substrate effuse, white. Mycelium superficial and immersed. Hyphae septate, branched, smooth, pale brown, 3–6 µm diam. Conidiophores distinct, mononematous, 4–12-septate, simple or occasionally branched near the base, erect, straight or flexuose, smooth, brown, becoming pale brown towards subhyaline apex, 32.5–155 × 3–7.5 µm; vesicle usually present at the apex, globose, pale brown to subhyaline, 5–9 × 4.5–8 µm; branches 1–2-septate, smooth, brown to pale brown, 10–35(–80) × 2.5–6 µm. Conidiogenous cells polyblastic, arise from creeping mycelium, from conidiophores or branches, intercalary or terminal, integrated, cylindrical, smooth, brown to pale brown; denticles, 0.8–2 × 0.7–2 µm. Conidia holoblastic, acropleurogenous, 10–15-septate, helical, smooth, hyaline, 11–18 µm diam., conidial filament 1–1.5 µm wide, coiled 2–3.5 times.

Other material examined: BRAZIL. BAHIA: Morro do Chápeu, on decaying twig, 12.II.2009, T.S. Santa Izabel, HUEFS 141556.

Comments: *Helicosporium vesiculiferum* is distinguished from other species in the genus by the presence of a vesicle at the conidiophore apex. Only four species produce conidia acropleurogenously: *H. gracile, H. griseum, H. guianense,* and *H. lumbricopsis. Helicosporium griseum* and *H. lumbricopsis* have conidiophores that are branched and often anastomosing, and can be differentiated easily from *H. vesiculiferum* (Berkeley 1874, Linder 1929). *Helicosporium guianense* possess branches along the tall conidiophores (Linder 1929). *Helicosporium gracile* is distinguished by yellow colony and absence of vesicles (Morgan 1892, Goos 1989).



Figs. 1–6. Light micrographs of *Helicosporium vesiculiferum* (from holotype). 1. General aspect on natural substrate; 2–3. Conidiophores with and without vesicles; 4–5. Detail of the conidiophore apex; 6. Conidium (Bars 1–3= 20 µm, 4–6= 5 µm)

Other species found in semi-arid region

Helicosporium aureum (Corda) Linder, Ann. Mo. bot. Gdn. 16: 279. 1929.

Tel.: *Acanthostigma scopulum* (Cooke & Peck) Peck, Bull. New York State Mus. 1: 22. 1887.

Colony yellow. Conidiophores 147–255 × 4–5.5 µm. Conidiogenous cells tooth-like or bladder-like, 4.5–13.5 × 2.5–3.5 µm. Conidia pleurogenous,

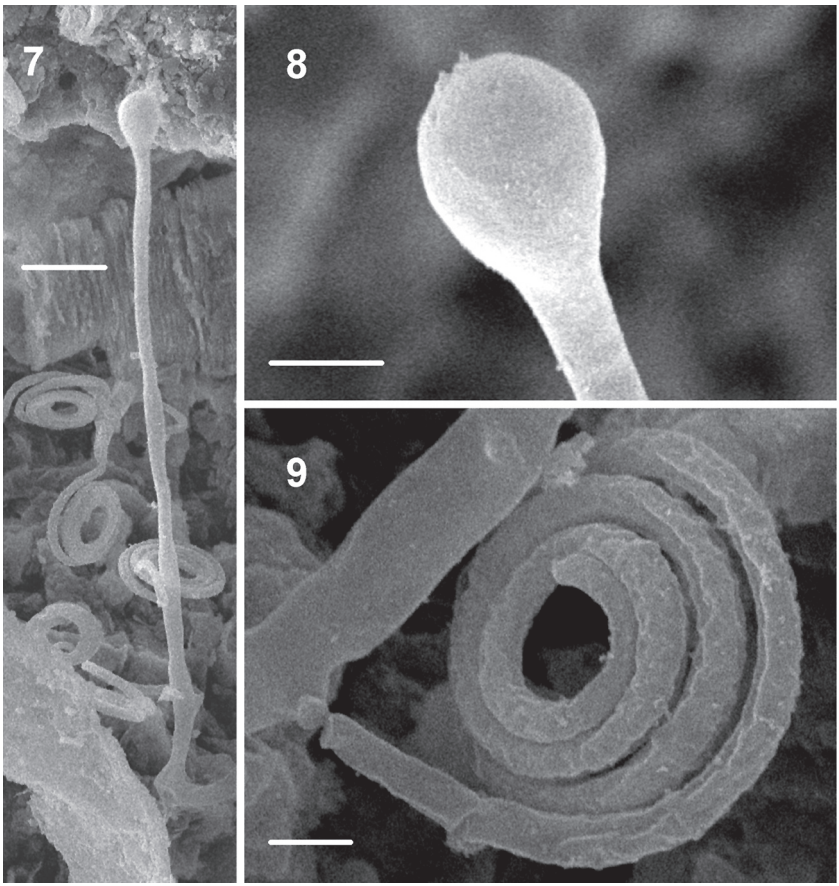


Fig. 7–9. Scanning Electron Microscope micrographs of *Helicosporium vesifuliferum*. 7. Conidiophore; 8. Vesicle; 9. Detail of the secession of conidium. (Bars fig. 7= 10 μm, 8= 3 μm, 9= 2 μm)

9–20-septate, 10.5–15μm diam., filament width 1–1.7 μm, number of coils 2.5–4 times.

Specimens examined: BRAZIL. Bahia: Pilão Arcado, on dead bark, 16.II.2007. A.C.R. Cruz, HUEFS 129346; Senhor do Bonfim, "Carrapichel", on decaying twig, 9.X.2006, A.C.R. Cruz, HUEFS 129347.

distribution: Argentina, Australia, Austria, Belize, Brazil, China, Czech Republic, formerly USSR, Japan, Pakistan, Panama, New Zealand, South Africa, USA (CBS 2009, Goos 1989, Farr & Rossman 2009, Morris 1978, Romero & Pildain 2004, Zhao et al. 2007).

Helicosporium aureum is similar to *H. decumbens*, *H. guianense* and *H. hiospiroides* B.S. Reddy et al. in possessing discrete, polyblastic conidio-

genous cells. *Helicosporium decumbens* and *H. hiospiroides* are distinguished by conidia diameter. *Helicosporium guianense* develops branches along its conidiophores. The Brazilian specimens are consistent with the descriptions given by Linder (1929), Goos (1989), and Zhao et al. (2007) regarding the absence of apical branches on conidiophores. *Helicosporium aureum* is recorded for the first time for Brazil.

Helicosporium gracile (Morgan) Linder, Ann. Missouri Bot. Gard. 16: 281. 1929.

Colony yellow. Conidiophores 25.5–150 × 3–6 µm. Conidiogenous cells tooth-like. Conidia acropleurogenous, 12–15-septate, 12–15µm diam., filament width 1–1.5 µm, number of coils 2–3.5 times.

Specimens examined: BRAZIL. Bahia: Morro do Chapéu, on dead leaves, 07.X.2008. T.S. Santa Izabel, HUEFS 141555.

distribution: Africa, Brazil, China, USA (Goos 1989, Linder 1929, Zhao et al. 2007).

The characteristics observed on the examined specimen are included within the morphological variation for the species (Linder 1929, Goos 1989, Zhao et al. 2007). *Helicosporium gracile* is closely related to *H. guianense, H. aureum,* and *H. virescens.* However, *H. guianense* differs from *H. gracile* by consistent branches along the conidiophores. *Helicosporium aureum* can be separated by bladder-like conidiogenous cells and larger conidiophores and *H. virescens* has tooth or bladder-like conidiogenous cells and larger conidiophores (Goos 1989, Zhao et al. 2007). This species is reported for the first time in South America.

Helicosporium griseum Berk. & M.A. Curtis, Grevillea 3: 51. 1874.
[non. *H. griseum* (Bonord.) Sacc. 1886]

Colony pinkish. Conidiophores 159–390 × 3–5 µm. Conidiogenous cells tooth-like. Conidia pleurogenous, 15–25-septate, 11.5–15 µm diam., filament width 1–1.5 µm, number of coils 3.5–4 times.

Specimens examined: BRAZIL. Bahia: Jeremoabo, on dead fruit, 18.I.2007, A.C.R. Cruz, HUEFS 129350; Senhor do Bonfim, on dead fruit, 28.IX.2006, A.C.R. Cruz, HUEFS 129351; on decaying twig, 28.IX.2006 A.C.R. Cruz, HUEFS 129352. Pernambuco, Buíque, on rotten leaf, 21.VIII.2006 A.C.R. Cruz, HUEFS 129353.

distribution: Australia, Belgium, Brazil, Canada, China, Costa Rica, Cuba, Czech Republic, formerly USSR, Germany, Hungary, Japan, Mexico, New Guinea, New Zealand, Panama, South Africa, Taiwan, USA (CBS 2009, Cooper 2005, Delgado-Rodriguez et al. 2002, Farr & Rossman 2009, Goos 1989, Heredia et al. 1995, Mercado-Sierra et al. 1997, Paulus et al. 2006, Révay 1998, Tsui et al. 2006, Zhao et al. 2007)

Helicosporium griseum is characterized by much branched conidiophores that frequently anastomose. The conidia are smaller in diameter than those cited by Linder (1929), Goos (1989), and Mercado-Sierra (1984) but are similar to

those reported by Zhao et al. (2007). *Helicosporium lumbricopsis* resembles *H. griseum* but is distinguished by the size of the conidia filament, diameter, and stouter conidiophores (Linder 1929, Zhao et al. 2007). This species was collected in Brazil under the name *H. griseum* (Bonord.) Sacc. and deposited at Centraalbureau voor Schimmelcultures (CBS) by R.F. Castañeda Ruiz (CBS 2009). According to Castañeda Ruiz (pers. comm.), the culture produces velvety colonies and anastomosing conidiophores and so is believed to represent *H. griseum* Berk. & M.A. Curtis.

Helicosporium nizamabadense P. Rag. Rao & D. Rao, Mycopathol. Mycol. Appl. 24: 34. 1964.

Colony white. Conidiophores 60–155 × 2–3.5 µm. Conidiogenous cells tooth-like. Conidia pleurogenous, 15–24 µm diam., filament width 1–3 µm, number of coils 3–4.5 times.

Specimens examined: BRAZIL. Bahia: Serra do Ramalho, on decaying leaves of unidentified dicotyledonous plant, 07.I.2008, S.M. Leão-Ferreira, HUEFS 136881.

distribution: Brazil, India (Rao & Rao 1964).

The examined material has a white colony and conidia are produced from distinct denticles on conidiogenous cells (Rao & Rao 1964). *Helicosporium dentophorum* G.Z. Zhao et al., *H. sympodiophorum* G.Z. Zhao et al., and *H. talbotii* Goos (Goos 1989, Zhao et al. 2007) have similar denticles. However, *H. dentophorum* differs by the presence of smaller conidiophores and bigger conidia, *H. sympodiophorum* has bigger conidiophores and conidia, and *H. talbotii* has conidia with narrower filaments. This is the first record for the American continent.

Helicosporium panacheum R.T. Moore, Mycologia 46: 92, 1954.

Colony white. Conidiophores 35–135 × 3–4.5 µm. Conidiogenous cells tooth-like. Conidia pleurogenous, multiseptate, 12–16 µm diam., filament width 1–1.5 µm, number of coils 2–3.5 times.

Specimens examined: BRAZIL. Bahia, Jeremoabo, "Mata das Pororocas", on dead bark, 16.I.2007, A.C.R. Cruz, HUEFS 129348; Senhor do Bonfim, "fazenda Passaginha", on dead bark, 5.X.2006, A.C.R. Cruz, HUEFS 129349.

distribution: Brazil, Canada, China, Japan, Mexico, Taiwan, USA, (Arias et al. 2000, CBS 2009, Chang 2001, Goos 1989, Moore 1954, Zhao et al. 2007).

The specimens examined have conidia smaller than in the original description (Moore 1954) but possess the typical characteristics of the species, having hyaline, branched conidiophores at apex and conidia that are white in mass. Zhao et al. (2007) described the same species with wider conidiophores. *Helicosporium panacheum* is reported for the first time in South America.

Helicosporium pannosum (Berk. & M.A. Curtis) R.T. Moore, Mycologia 49: 582. 1957.

Tel.: *Thaxteriella helicoma* (W. Phillips & Plowr.) J.L. Crane, Shearer & M.E. Barr, Can. J. Bot. 76: 610. 1998.

Colony brown. Conidiophores 95–215 × 6–7.5 µm. Conidiogenous cells tooth-like. Conidia pleurogenous, 25–50-septate, 34.5–45 µm diam., filament width 3–4.5 µm, number of coils 1.5–4 times.

Specimens examined: BRAZIL. Bahia, Senhor do Bonfim, "fazenda Passaginha", on decaying fruit, 5.X.2006, A.C.R. Cruz, HUEFS 129354.

distribution: Argentina, Australia, Brazil, Chile, China, Cuba, Guiana, Haiti Japan, New Zealand, Papua New Guinea, Seychelles Islands, Sri Lanka, Suriname, Tanzania, Thailand, United Kingdom, USA (Barr 1980, CBS 2009, Delgado-Rodríguez et al. 2002, Farr & Rossman 2009, Hughes 1978, Linder 1929, Matsushima 1975, Pinruan et al. 2007, Romero 1983, Sivanesan 1984, Tsui et al. 2001).

Helicosporium pannosum has a very wide widest conidia filament. The conidia of the material examined are smooth, but the specimens examined by Goos (1989) are minutely echinulate. *Helicosporium pannosum* is close to *H. hongkongense* K.M. Tsui et al. with a similar conidia filament width, but its conidiophores are always simple and conidia are subhyaline to yellowish (Tsui et al. 2001). Conidia are smaller than material studied by Zhao et al. (2007).

Helicosporium virescens (Pers.) Sivan., Bitunicate Ascomycetes and their Anamorphs (Vaduz): 591. 1984.

Tel.: *Tubeufia cerea* (Berk. & M.A. Curtis) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1, 128: 562. 1919.

Colony yellow. Conidiophores 125–375 × 3–6 µm. Conidiogenous cells tooth-like. Conidia pleurogenous, multiseptate, 7.5–13 µm diam., filament width 0.75–1.2 µm, number of coils 2–3.5 times.

Specimens examined: BRAZIL. Bahia: Campo Formoso, "Mata da Esterzinha", on decaying twig, 19.IX.2006, A.C.R. Cruz, HUEFS 129355; Jeremoabo, on dead bark, 17.I.2007 A.C.R. Cruz, HUEFS 129356; Senhor do Bonfim, "Carrapichel", on decaying spathe of *Syagrus coronata* (Mart.) Becc., 9.X.2006, A.C.R. Cruz, HUEFS 129357; Senhor do Bonfim, "Serra da Maravilha", on dead bark, 11.X.2006, A.C.R. Cruz, HUEFS 129358. Pernambuco, Buíque, on rotten leaf, 11.X.2006, A.C.R. Cruz, HUEFS 129359.

distribution: Austria, Belgium, Brazil, Canada, China, Costa Rica, Cuba, France, Germany, Guiana, Hungary, India, Lithuania, Mexico, Netherlands, Poland, Republic of Belarus, Thailand, United Kingdom, USA (Barr 1980, Farr & Rossman 2009, Goos 1989, Heredia et al. 1995, Linder 1929, Mercado-Sierra et al. 1997, 1998, Révay 1998, Sivanesan 1984, Tsui et al. 2006, Yurchenko 2001, Zhao et al. 2007).

The characteristics observed on the specimens examined are included within the morphological variation of the species (Goos 1989, Zhao et al. 2007). *Helicosporium virescens* differs from *H. aureum* and *H. murinum* Goos by conidiophores that are mostly unbranched. This species was found for the first time for Brazil.

Key to species of *Helicosporium*

1a. Conidia borne acrogenously 2
1b. Conidia borne acropleurogenously 3
1c. Conidia borne pleurogenously 5

2a. (1a). Conidiophores less than 40 µm long, conidia coiled 2–2.25 times ..... *H. dentophorum*
2b. Conidiophores more than 40 µm long, conidia coiled 3.5–4 times ..... *H. sympodiophorum*

3a. (1b). Conidia more than 20 µm diam ..... *H. panacheum*
3b. Conidia less than 20 µm diam 4

4a. (3b). Vesicle absent at the apex of conidiophores ..... *H. gracile*
4b. Vesicles usually present at the apex of conidiophores ..... *H. vesiculiferum*

5a. (1c). Conidiogenous cells bladder-like ..... *H. hiospiroides*
5b. Conidiogenous cells tooth-like and bladder-like 6
5c. Conidiogenous cells tooth-like 9

6a. (5b). Conidia less than 10 µm diam ..... *H. decumbens*
6b. Conidia more than 10 µm diam 7

7a. (6b). Conidiophores mostly unbranched ..... *H. virescens*
7b. Conidiophores mostly branched 8

8a. (7b). Conidiophores often branched and entangled above ..... *H. aureum*
8b. Conidiophores branched along the mains axis ..... *H. guianense*

9a. (5c). Two kinds of conidiophores (macro and micro) ..... *H. indicum*
9b. One kind of conidiophores 10

10a. (9b). Conidiophores consistently branched, anastomosing frequently 11
10b. Conidiophores simple, sparsely branched, or occasionally anastomosing 12

11a. (10a). Conidiophores subhyaline to dark brown, ascending or more or less erect ..... *H. griseum*
11b. Conidiophores dark, erect, at first simple, later branching ..... *H. lumbricopsis*

12a. (10b). Conidiophores mostly colorless, rarely pale brown 13
12b. Conidiophores pale brown to brown 14

13a. (12a). Conidia 10–15 µm diam ..... *H. pallidum*
13b. Conidia 18–28 µm diam ..... *H. nizamabadense*

14a. (12b). Conidia less than 24 µm diam 15
14b. Conidia mostly more than 24 µm diam 18

15a. (14a). Conidiophores arising from repent mycelium ..... *H. talbotii*
15b. Conidiophores not as above 16

16a. (15b). Conidial filaments 1–1.5 µm thick ..... *H. murinum*
16b. Conidial filaments 1.5–2.7 µm thick 17

17a. (16b). Conidiophores mostly simple, rarely branched, conidial filaments 1.5–2 µm thick. . . . . *H. abuense*

17b. Conidiophores branched from basal parts, conidial filaments 2–2.7 µm thick. . . . . *H. phragmitis*

18a. (14b). Conidia more than 60 µm diam . . . . . *H. raghuveeri*

18b. Conidia less than 60 µm diam. 19

19a. (18b). Conidiophores simple, conidia subhyaline to yellowish . . . *H. hongkongense*

19b. Conidiophores simple or branched from basal parts, conidia subhyaline to pale brown. . . . . *H. pannosum*

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